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(57) Abstract

HABC7 polpeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing HABC7 polypeptides and polynucleotides in the design of protocols for the treatment of cancer, autoimmune disease, Addison's disease, microsomal disorders, and IDDM, among others, and diagnostic assays for such conditions.

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A HUMAN ABC TRANSPORTER-7 (HABC7) GENE

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to the ABC transporter gene family, hereinafter referred to as HABC7. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

The murine ABC7 transporter protein is a typical half-transporter, having six transmembrane domains in the N-terminal region and an ATP binding cassette (ABC motif) in the C-terminal region. It functions as a membrane pump required for other half-transporters such as TAP1 and TAP2 to form either homodimers or heterodimers. This indicates that the ABC transporter gene family has an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further members of the ABC transporter gene family which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, cancer, autoimmune disease, Addison's disease, microsomal disorders, and IDDM.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to HABC7 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such HABC7 polypeptides and polynucleotides. Such uses include the treatment of cancer, autoimmune disease, Addison's disease, microsomal disorders, and IDDM, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with HABC7 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate HABC7 activity or levels.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"HABC7" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 or an allelic variant thereof.

"HABC7 activity or HABC7 polypeptide activity" or "biological activity of the HABC7 or HABC7 polypeptide" refers to the metabolic or physiologic function of said HABC7 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said HABC7.

"HABC7 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than

the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many

regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al.*, *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990) 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5 or 3

terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Invention

In one aspect, the present invention relates to HABC7 polypeptides (or HABC7 proteins). The HABC7 polypeptides include the polypeptide of SEQ ID NO:2; as well as polypeptides comprising the amino acid sequence of SEQ ID NO: 2; and polypeptides comprising the amino acid sequence which have at least 84% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within HABC7 polypeptides are polypeptides having the amino acid sequence which have at least 84% identity to the polypeptide having the amino acid sequence of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and still more preferably at least 95% identity to SEQ ID NO:2. Furthermore, those with at least 97-99% are highly preferred. Preferably HABC7 polypeptide exhibit at least one biological activity of HABC7.

The HABC7 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the HABC7 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned HABC7 polypeptides. As with HABC7 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of HABC7 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of HABC7 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate HABC7 activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the HABC7, including antigenic activity. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The HABC7 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to HABC7 polynucleotides. HABC7 polynucleotides include isolated polynucleotides which encode the HABC7 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, HABC7 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding a HABC7 polypeptide of SEQ ID NO: 2, and polynucleotide having the particular sequence of SEQ ID NO:1. HABC7 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 82% identity over its entire length to a nucleotide sequence encoding the HABC7 polypeptide of SEQ ID NO:2, and a polynucleotide comprising a nucleotide sequence that is at least 82% identical to of SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under HABC7 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such HABC7 polynucleotides.

HABC7 of the invention is structurally related to other proteins of the ABC transporter gene family, as shown by the results of sequencing the cDNA of Table 1 (SEQ ID NO:1) encoding human HABC7. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 9 to 2264) encoding a polypeptide of 752 amino acids of SEQ ID NO:2. The amino acid sequence of Table 2 (SEQ ID NO:2) has about 84% identity (using FASTA) in 752 amino acid residues with mouse ABC transporter-7 (S.Savary et al.,Genomics,41:275-278,1997). The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 81.4% identity (using FASTA) in 2384 nucleotide residues with mouse ABC transporter-7 (S.Savary et al.,Genomics,41:275-278,1997). Thus, HABC7 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

Table 1[a]

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GGCTCAAGATGGCGCTGCTCGCGATGCATTCTTGGCGCTGGGCGGCCGCGGCGGCTGCTTTCGAAAAGC
GCCGGCACTCCGCGATTCTGATCCGGCCTTTAGTCTCTGTTAGCGGCTCAGGTCCGCAGTGGAGGCCAC
ATCAACTCGGCGCCTTGGGAACCGCTCGAGCCTACCAGATTCCAGAGTCATTAAAAAGTATCACATGGC
AGAGATTGGGAAAAGGCAATTCAGGACAGTTCTTAGATGCTGCAAAGGCTCTCCAGGTATGGCCACTGA
TAGAAAAGAGGACATGTTGGCATGGTCATGCAGGAGGAGGACTCCACACAGACCCAAAAGAAGGGTTAA
AAGATGTTGATACTCGGAAAATCATAAAAGCAATGCTTTCTTATGTGTGGCCCAAAGACAGGCCAGATC
TACGAGCTAGAGTTCCCATTTCGCTGGGATTTTTGGGTGGTGCAAAGGCCATGAATATTGTGGTTCCCT
TCATGTTTAAATATGCTGTAGACAGCCTCAACCAGATGTCGGGAAACATGCTGAACCTGAGTGATGCAC
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CAAATACAGTTGCAACCATGGCAACAGCAGTTCTGATTGGCTATGGTGTATCAAGAGCTGGAGCTGCTT
TTTTTAACGAAGTTCGAAATGCAGTATTTGGCAAGGTAGCCCAGAATTCAATCCGAAGAATAGCCAAAA
ATGTCTTTCTCCATCTTCACAACCTGGATCTGGGTTTTCACCTGAGCAGACAGACGGGAGCTTTATCTA
AGGCTATTGACAGAGGAACAAGGGGTATCAGTTTTGTCCTGAGTGCTTTGGTATTTAATCCTCTCCCTA
ATCATGTTGAAGTGATGCTTCTTGTCAGTGGTGTTTTGTATTACAAATGCTGTGCCCAGTTGCTTGGTA
ACCTTGGAACACTTGGTACATACACAGCATTCACAGTTGCAGTCACACGGTGGAGAACTAGATTTAGAT
TGGAAATTGACCAAGCAGATAATGATGCAGGTAATGCTGCTATAGACTCACTGCTGAATTATGAAACTG
TGAAGTATTTTAATAATGAAAGATATGAAGCACAGAGATATGATGGATTCTTGAAGACGTATGAGACTG
CTTCATTGAAAAGTACCTCTACTCTGGCTATGCTGAACTTTGGTCAAAGTGCTATTTTCAGTGTCGGTT
TAACAGCTATAATGGTGCTCGCCAGTCAGGGAATTGTGGCAGGTACCCTTACTGTTGGAGATCTAGTAA
TGGTGAATGGACTGCTTTTTCAGCTTTCATTACCCCTGAACTTTCTGGGAACTGTATATAGAGAGACTA
GACAAGCACTCATAGATATGAACACCTTGTTTACTCTACTCAAGGTAGACACCCAAATTAAAGACAAAG
TGATGGCATCTCCCCTTCAGATCACACCACAGACAGCTACCGTGGCCTTTGATAATGTGCATTTTGAAT
ACATTGAGGGCCAGAAAGTCCTTAGTGGAATATCCTTTGAAGTCCCTGCAGGAAAGAAAGTGGCCATTG
TAGGAGGTAGTGGGTCAGGGAAAAGCACAATAGTGAGGCTATTATTTCGCTTCTATGAGCCTCAAAAGG
GTAGCATTTATCTTGCTGGTCAAAATATACAAGATGTGAGCCTGGAAAGCCTTCGGAGGGCAGTGGGAG
TGGTACCTCAGGATGCTGTCCTCTTCCATAATACTATTTATTACAACCTCTTATATGGAAACATCAGTG
CTTCACCTGAGGAAGTGTATGCAGTGGCAAAATTAGCTGGACTTCATGATGCAATTCTTCGAATGCCAC
ATGGATATGACACCCAAGTAGGGGAACGAGGACTCAAGCTTTCAGGAGGAGAAAAGCAAAGAGTAGCAA
TTGCAAGAGCCATTTTGAAGGACCCCCCAGTCATACTCTACGATGAAGCTACTTCATCGTTAGATTCGA
TTACTGAAGAGACTATTCTTGGTGCCATGAAGGATGTGGTCAAACACAGAACTTCTATTTTCATTGCAC
ACAGATTGTCAACAGTGGTTGATGCAGATGAAATCATTGTCTTGGATCAGGGTAAGGTAGCCGAACGTG
GTACCCACCATGGTTTGCTTGCTAACCCTCATAGTATCTATTCAGAAATGTGGCATACACAGAGCAGCC
GTGTGCAGAACCATGATAACCCCAAATGGGAAGCAAAGAAAGAAAATATATCCAAAGAGGAGGAAAGAA
AGAAACTACAAGAAGAAATTGTCAATAGTGTGAAAGGCTGTGGAAACTGTTCGTGCTAAGTCACATAAG
ACATTTTCTTTTTTTGTTGTTTTGGACTACATATTTGCACTGAAGCAGAATTGTTTTATTAAAAAAATC
ATACATTCAAAAAAAAAAAAAAAAAAAAAAAAAAAA

[a] A nucleotide sequence of a human HABC7 (SEQ ID NO: 1).

Table 2[b]

MALLAMHSWRWAAAAAAFEKRRHSAILIRPLVSVSGSGPQWRPHQLGALGTARAYQIPESLKSITWQRL
GKGNSGQFLDAAKALQVWPLIEKRTCWHGHAGGGLHTDPKEGLKDVDTRKIIKAMLSYVWPKDRPDLRA
RVPISLGFLGGAKAMNIVVPFMFKYAVDSLNQMSGNMLNLSDAPNTVATMATAVLIGYGVSRAGAAFFN
EVRNAVFGKVAQNSIRRIAKNVFLHLHNLDLGFHLSRQTGALSKAIDRGTRGISFVLSALVFNPLPNHV
EVMLLVSGVLYYKCCAQLLGNLGTLGTYTAFTVAVTRWRTRFRLEIDQADNDAGNAAIDSLLNYETVKY
FNNERYEAQRYDGFLKTYETASLKSTSTLAMLNFGQSAIFSVGLTAIMVLASQGIVAGTLTVGDLVMVN
GLLFQLSLPLNFLGTVYRETRQALIDMNTLFTLLKVDTQIKDKVMASPLQITPQTATVAFDNVHFEYIE
GQKVLSGISFEVPAGKKVAIVGGSGSGKSTIVRLLFRFYEPQKGSIYLAGQNIQDVSLESLRRAVGVVP
QDAVLFHNTIYYNLLYGNISASPEEVYAVAKLAGLHDAILRMPHGYDTQVGERGLKLSGGEKQRVAIAR
AILKDPPVILYDEATSSLDSITEETILGAMKDVVKHRTSIFIAHRLSTVVDADEIIVLDQGKVAERGTH

HGLLANPHSIYSEMWHTQSSRVQNHDNPKWEAKKENISKEEERKKLQEEIVNSVKGCGNCSC

[b] An amino acid sequence of a human HABC7 (SEQ ID NO: 2).

One polynucleotide of the present invention encoding HABC7 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human cord blood using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al.*, *Nature*, (1992) *355*:632-634; Adams, M.D., *et al.*, *Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding HABC7 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 9 to 2264 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of HABC7 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding HABC7 variants comprise the amino acid sequence HABC7 polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at

least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding HABC7 polypeptide and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to the HABC7 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding HABC7 polypeptide, including homologs and orthologs from species other than human, comprises the steps of screening an appropriate library under stingent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Thus in another aspect, HABC7 polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof. Also included with HABC7 polypeptides are polypeptide comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or, alternatively, conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation

systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the HABC7 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If HABC7 polypeptide is secreted into the medium,

the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.
HABC7 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of HABC7 polynucleotides for use as diagnostic reagents. Detection of a mutated form of HABC7 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of HABC7. Individuals carrying mutations in the HABC7 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled HABC7 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising HABC7 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to cancer, autoimmune disease, Addison's disease, microsomal disorders, and IDDM through detection of mutation in the HABC7 gene by the methods described.

In addition, cancer, autoimmune disease, Addison's disease, microsomal disorders, and IDDM, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of HABC7 polypeptide or HABC7 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an HABC7 polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagonostic kit for a disease or suspectability to a disease, particularly cancer, autoimmune disease, Addison's disease, microsomal disorders, and IDDM, which comprises:

(a) a HABC7 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof ;

(b) a nucleotide sequence complementary to that of (a);

(c) a HABC7 polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or

(d) an antibody to a HABC7 polypeptide, preferably to the polypeptide of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease. The HABC7 gene has been localized to the X chromosome on q21.3.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the HABC7 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the HABC7 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against HABC7 polypeptides may also be employed to treat cancer, autoimmune disease, Addison's disease, microsomal disorders, and IDDM, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with HABC7 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from cancer, autoimmune disease, Addison's disease, microsomal disorders, and IDDM, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering HABC7 polypeptide via a vector directing expression of

HABC7 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a HABC7 polypeptide wherein the composition comprises a HABC7 polypeptide or HABC7 gene. The vaccine formulation may further comprise a suitable carrier. Since HABC7 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

The HABC7 polypeptide of the present invention may be employed in a screening process for compounds which activate (agonists) or inhibit activation of (antagonists, or otherwise called inhibitors) the HABC7 polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess identify agonist or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These agonists or antagonists may be natural or modified substrates, ligands, receptors, enzymes, etc., as the case may be, of the polypeptide of the present invention; or may be structural or functional mimetics of the polypeptide of the present invention. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

HABC7 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate HABC7 polypeptide on the one hand and which can inhibit the function of HABC7 polypeptide on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as cancer, autoimmune disease, Addison's disease, microsomal disorders, and IDDM. Antagonists may be employed for a

variety of therapeutic and prophylactic purposes for such conditions as cancer, autoimmune disease, Addison's disease, microsomal disorders, and IDDM.

In general, such screening procedures may involve using appropriate cells which express the HABC7 polypeptide or respond to HABC7 polypeptide of the present invention. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells which express the HABC7 polypeptide (or cell membrane containing the expressed polypeptide) or respond to HABC7 polypeptide are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The ability of the cells which were contacted with the candidate compounds is compared with the same cells which were not contacted for HABC7 activity.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the HABC7 polypeptide is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the HABC7 polypeptide, using detection systems appropriate to the cells bearing the HABC7 polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

Further, the assays may simply comprise the steps of mixing a candidate compound with a solution containing a HABC7 polypeptide to form a mixture, measuring HABC7 activity in the mixture, and comparing the HABC7 activity of the mixture to a standard.

The HABC7 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of HABC7 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of HABC7 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of HABC7 (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The HABC7 protein may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. These include, but are not limited to, ligand binding and crosslinking assays in which the HABC7 is labeled with a radioactive isotope (eg 125I), chemically modified (eg biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. In addition to being used for

purification and cloning of the receptor, these binding assays can be used to identify agonists and antagonists of HABC7 which compete with the binding of HABC7 to its receptors, if any. Standard methods for conducting screening assays are well understood in the art.

Examples of potential HABC7 polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, enzymes, etc., as the case may be, of the HABC7 polypeptide, e.g., a fragment of the ligands, substrates, receptors, enzymes, etc.; or small molecules which bind to the polypetide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for HABC7 polypeptides; or compounds which decrease or enhance the production of HABC7 polypeptides, which comprises:
(a) a HABC7 polypeptide, preferably that of SEQ ID NO:2;
(b) a recombinant cell expressing a HABC7 polypeptide, preferably that of SEQ ID NO:2;
(c) a cell membrane expressing a HABC7 polypeptide; preferably that of SEQ ID NO: 2; or
(d) antibody to a HABC7 polypeptide, preferably that of SEQ ID NO: 2.
It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions such as, cancer, autoimmune disease, Addison's disease, microsomal disorders, and IDDM, related to both an excess of and insufficient amounts of HABC7 polypeptide activity.

If the activity of HABC7 polypeptide is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit the function of the HABC7 polypeptide, such as, for example, by blocking the binding of ligands, substrates, receptors, enzymes, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of HABC7 polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous HABC7 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the HABC7 polypeptide.

In still another approach, expression of the gene encoding endogenous HABC7 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example,

O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al., Nucleic Acids Res* (1979) 6:3073; Cooney *et al., Science* (1988) 241:456; Dervan *et al., Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of HABC7 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates HABC7 polypeptide, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of HABC7 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of HABC7 polypeptides in combination with a suitable pharmaceutical carrier.

Formulation and Administration

Peptides, such as the soluble form of HABC7 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or

intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 82% identity over its entire length to a nucleotide sequence encoding the HABC7 polypeptide of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.

2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO:1 encoding the HABC7 polypeptide of SEQ ID NO2.

3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 82% identical to that of SEQ ID NO: 1 over its entire length.

4. The polynucleotide of claim 3 which is the polynucleotide of SEQ ID NO: 1.

5. The polynucleotide of claim 1 which is DNA or RNA.

6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a HABC7 polypeptide comprising an amino acid sequence, which has at least 84% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.

7. A host cell comprising the expression system of claim 6.

8. A process for producing a HABC7 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.

9. A process for producing a cell which produces a HABC7 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a HABC7 polypeptide.

10. A HABC7 polypeptide comprising an amino acid sequence which is at least 84% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.

11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.

12. An antibody immunospecific for the HABC7 polypeptide of claim 10.

13. A method for the treatment of a subject in need of enhanced activity or expression of HABC7 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an agonist to said polypeptide; and/or

(b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 82% identity to a nucleotide sequence encoding the HABC7 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said polypeptide activity *in vivo*.

14. A method for the treatment of a subject having need to inhibit activity or expression of HABC7 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said polypeptide; and/or

(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said polypeptide; and/or

(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said polypeptide for its ligand, substrate , or receptor.

15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of HABC7 polypeptide of claim 10 in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said HABC7 polypeptide in the genome of said subject; and/or

(b) analyzing for the presence or amount of the HABC7 polypeptide expression in a sample derived from said subject.

16. A method for identifying compounds which inhibit (antagonize) or agonize the HABC7 polypeptide of claim 10 which comprises:

(a) contacting a candidate compound with cells which express the HABC7 polypeptide (or cell membrane expressing HABC7 polypeptide) or respond to HABC7 polypeptide; and

(b) observing the binding, or stimulation or inhibition of a functional response; or comparing the ability of the cells (or cell membrane) which were contacted with the candidate compounds with the same cells which were not contacted for HABC7 polypeptide activity.

17. An agonist identified by the method of claim 16.

18. An antagonist identified by the method of claim 16.

19. A recombinant host cell produced by a method of Claim 9 or a membrane thereof expressing a HABC7 polypeptide.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: ZHANG, QING-HUA

(ii) TITLE OF THE INVENTION: A HUMAN ABC TRANSPORTER-7 (HABC7) GENE

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: RATNER & PRESTIA
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(C) CITY: VALLEY FORGE
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19482

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE:
(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: PRESTIA, PAUL F
(B) REGISTRATION NUMBER: 23,031
(C) REFERENCE/DOCKET NUMBER: GP-70305

(ix) TELECOMMUNICATION INFORMATION:

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(B) TELEFAX: 610-407-0701
(C) TELEX: 846169

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2384 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCTCAAGAT GGCGCTGCTC GCGATGCATT CTTGGCGCTG GGCGGCCGCG GCGGCTGCTT 60
TCGAAAAGCG CCGGCACTCC GCGATTCTGA TCCGGCCTTT AGTCTCTGTT AGCGGCTCAG 120
GTCCGCAGTG GAGGCCACAT CAACTCGGCG CCTTGGGAAC CGCTCGAGCC TACCAGATTC 180
CAGAGTCATT AAAAAGTATC ACATGGCAGA GATTGGGAAA AGGCAATTCA GGACAGTTCT 240
TAGATGCTGC AAAGGCTCTC CAGGTATGGC CACTGATAGA AAAGAGGACA TGTTGGCATG 300
GTCATGCAGG AGGAGGACTC CACACAGACC CAAAAGAAGG GTTAAAAGAT GTTGATACTC 360
GGAAAATCAT AAAAGCAATG CTTTCTTATG TGTGGCCCAA AGACAGGCCA GATCTACGAG 420
CTAGAGTTCC CATTTCGCTG GGATTTTTGG GTGGTGCAAA GGCCATGAAT ATTGTGGTTC 480
CCTTCATGTT TAAATATGCT GTAGACAGCC TCAACCAGAT GTCGGGAAAC ATGCTGAACC 540
TGAGTGATGC ACCAAATACA GTTGCAACCA TGGCAACAGC AGTTCTGATT GGCTATGGTG 600
TATCAAGAGC TGGAGCTGCT TTTTTTAACG AAGTTCGAAA TGCAGTATTT GGCAAGGTAG 660
CCCAGAATTC AATCCGAAGA ATAGCCAAAA ATGTCTTTCT CCATCTTCAC AACCTGGATC 720
TGGGTTTTCA CCTGAGCAGA CAGACGGGAG CTTTATCTAA GGCTATTGAC AGAGGAACAA 780
GGGGTATCAG TTTTGTCCTG AGTGCTTTGG TATTTAATCC TCTCCCTAAT CATGTTGAAG 840
TGATGCTTCT TGTCAGTGGT GTTTTGTATT ACAAATGCTG TGCCCAGTTG CTTGGTAACC 900
TTGGAACACT TGGTACATAC ACAGCATTCA CAGTTGCAGT CACACGGTGG AGAACTAGAT 960
TTAGATTGGA AATTGACCAA GCAGATAATG ATGCAGGTAA TGCTGCTATA GACTCACTGC 1020
TGAATTATGA AACTGTGAAG TATTTTAATA ATGAAAGATA TGAAGCACAG AGATATGATG 1080
GATTCTTGAA GACGTATGAG ACTGCTTCAT TGAAAAGTAC CTCTACTCTG GCTATGCTGA 1140
ACTTTGGTCA AAGTGCTATT TTCAGTGTCG GTTTAACAGC TATAATGGTG CTCGCCAGTC 1200
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TTCAGCTTTC ATTACCCCTG AACTTTCTGG GAACTGTATA TAGAGAGACT AGACAAGCAC 1320
TCATAGATAT GAACACCTTG TTTACTCTAC TCAAGGTAGA CACCCAAATT AAAGACAAAG 1380
TGATGGCATC TCCCCTTCAG ATCACACCAC AGACAGCTAC CGTGGCCTTT GATAATGTGC 1440
ATTTTGAATA CATTGAGGGC CAGAAAGTCC TTAGTGGAAT ATCCTTTGAA GTCCCTGCAG 1500
GAAAGAAAGT GGCCATTGTA GGAGGTAGTG GGTCAGGGAA AAGCACAATA GTGAGGCTAT 1560
TATTTCGCTT CTATGAGCCT CAAAAGGGTA GCATTTATCT TGCTGGTCAA AATATACAAG 1620
ATGTGAGCCT GGAAAGCCTT CGGAGGGCAG TGGGAGTGGT ACCTCAGGAT GCTGTCCTCT 1680

TCCATAATAC TATTTATTAC AACCTCTTAT ATGGAAACAT CAGTGCTTCA CCTGAGGAAG 1740
TGTATGCAGT GGCAAAATTA GCTGGACTTC ATGATGCAAT TCTTCGAATG CCACATGGAT 1800
ATGACACCCA AGTAGGGGAA CGAGGACTCA AGCTTTCAGG AGGAGAAAAG CAAAGAGTAG 1860
CAATTGCAAG AGCCATTTTG AAGGACCCCC CAGTCATACT CTACGATGAA GCTACTTCAT 1920
CGTTAGATTC GATTACTGAA GAGACTATTC TTGGTGCCAT GAAGGATGTG GTCAAACACA 1980
GAACTTCTAT TTTCATTGCA CACAGATTGT CAACAGTGGT TGATGCAGAT GAAATCATTG 2040
TCTTGGATCA GGGTAAGGTA GCCGAACGTG GTACCCACCA TGGTTTGCTT GCTAACCCTC 2100
ATAGTATCTA TTCAGAAATG TGGCATACAC AGAGCAGCCG TGTGCAGAAC CATGATAACC 2160
CCAAATGGGA AGCAAAGAAA GAAAATATAT CCAAAGAGGA GGAAAGAAAG AAACTACAAG 2220
AAGAAATTGT CAATAGTGTG AAAGGCTGTG GAAACTGTTC GTGCTAAGTC ACATAAGACA 2280
TTTTCTTTTT TTGTTGTTTT GGACTACATA TTTGCACTGA AGCAGAATTG TTTTATTAAA 2340
AAAATCATAC ATTCAAAAAA AAAAAAAAAA AAAAAAAAAA AAAA 2384

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 752 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Leu Leu Ala Met His Ser Trp Arg Trp Ala Ala Ala Ala Ala
1 5 10 15
Ala Phe Glu Lys Arg Arg His Ser Ala Ile Leu Ile Arg Pro Leu Val
20 25 30
Ser Val Ser Gly Ser Gly Pro Gln Trp Arg Pro His Gln Leu Gly Ala
35 40 45
Leu Gly Thr Ala Arg Ala Tyr Gln Ile Pro Glu Ser Leu Lys Ser Ile
50 55 60
Thr Trp Gln Arg Leu Gly Lys Gly Asn Ser Gly Gln Phe Leu Asp Ala
65 70 75 80
Ala Lys Ala Leu Gln Val Trp Pro Leu Ile Glu Lys Arg Thr Cys Trp
85 90 95
His Gly His Ala Gly Gly Gly Leu His Thr Asp Pro Lys Glu Gly Leu
100 105 110
Lys Asp Val Asp Thr Arg Lys Ile Ile Lys Ala Met Leu Ser Tyr Val
115 120 125
Trp Pro Lys Asp Arg Pro Asp Leu Arg Ala Arg Val Pro Ile Ser Leu
130 135 140
Gly Phe Leu Gly Gly Ala Lys Ala Met Asn Ile Val Val Pro Phe Met
145 150 155 160

Phe Lys Tyr Ala Val Asp Ser Leu Asn Gln Met Ser Gly Asn Met Leu
165 170 175
Asn Leu Ser Asp Ala Pro Asn Thr Val Ala Thr Met Ala Thr Ala Val
180 185 190
Leu Ile Gly Tyr Gly Val Ser Arg Ala Gly Ala Ala Phe Phe Asn Glu
195 200 205
Val Arg Asn Ala Val Phe Gly Lys Val Ala Gln Asn Ser Ile Arg Arg
210 215 220
Ile Ala Lys Asn Val Phe Leu His Leu His Asn Leu Asp Leu Gly Phe
225 230 235 240
His Leu Ser Arg Gln Thr Gly Ala Leu Ser Lys Ala Ile Asp Arg Gly
245 250 255
Thr Arg Gly Ile Ser Phe Val Leu Ser Ala Leu Val Phe Asn Pro Leu
260 265 270
Pro Asn His Val Glu Val Met Leu Leu Val Ser Gly Val Leu Tyr Tyr
275 280 285
Lys Cys Cys Ala Gln Leu Leu Gly Asn Leu Gly Thr Leu Gly Thr Tyr
290 295 300
Thr Ala Phe Thr Val Ala Val Thr Arg Trp Arg Thr Arg Phe Arg Leu
305 310 315 320
Glu Ile Asp Gln Ala Asp Asn Asp Ala Gly Asn Ala Ala Ile Asp Ser
325 330 335
Leu Leu Asn Tyr Glu Thr Val Lys Tyr Phe Asn Asn Glu Arg Tyr Glu
340 345 350
Ala Gln Arg Tyr Asp Gly Phe Leu Lys Thr Tyr Glu Thr Ala Ser Leu
355 360 365
Lys Ser Thr Ser Thr Leu Ala Met Leu Asn Phe Gly Gln Ser Ala Ile
370 375 380
Phe Ser Val Gly Leu Thr Ala Ile Met Val Leu Ala Ser Gln Gly Ile
385 390 395 400
Val Ala Gly Thr Leu Thr Val Gly Asp Leu Val Met Val Asn Gly Leu
405 410 415
Leu Phe Gln Leu Ser Leu Pro Leu Asn Phe Leu Gly Thr Val Tyr Arg
420 425 430
Glu Thr Arg Gln Ala Leu Ile Asp Met Asn Thr Leu Phe Thr Leu Leu
435 440 445
Lys Val Asp Thr Gln Ile Lys Asp Lys Val Met Ala Ser Pro Leu Gln
450 455 460
Ile Thr Pro Gln Thr Ala Thr Val Ala Phe Asp Asn Val His Phe Glu
465 470 475 480
Tyr Ile Glu Gly Gln Lys Val Leu Ser Gly Ile Ser Phe Glu Val Pro
485 490 495
Ala Gly Lys Lys Val Ala Ile Val Gly Gly Ser Gly Ser Gly Lys Ser
500 505 510

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Thr Ile Val Arg Leu Leu Phe Arg Phe Tyr Glu Pro Gln Lys Gly Ser
        515                 520                 525
Ile Tyr Leu Ala Gly Gln Asn Ile Gln Asp Val Ser Leu Glu Ser Leu
    530                 535                 540
Arg Arg Ala Val Gly Val Val Pro Gln Asp Ala Val Leu Phe His Asn
545                 550                 555                 560
Thr Ile Tyr Tyr Asn Leu Leu Tyr Gly Asn Ile Ser Ala Ser Pro Glu
                565                 570                 575
Glu Val Tyr Ala Val Ala Lys Leu Ala Gly Leu His Asp Ala Ile Leu
            580                 585                 590
Arg Met Pro His Gly Tyr Asp Thr Gln Val Gly Glu Arg Gly Leu Lys
        595                 600                 605
Leu Ser Gly Gly Glu Lys Gln Arg Val Ala Ile Ala Arg Ala Ile Leu
    610                 615                 620
Lys Asp Pro Pro Val Ile Leu Tyr Asp Glu Ala Thr Ser Ser Leu Asp
625                 630                 635                 640
Ser Ile Thr Glu Glu Thr Ile Leu Gly Ala Met Lys Asp Val Val Lys
                645                 650                 655
His Arg Thr Ser Ile Phe Ile Ala His Arg Leu Ser Thr Val Val Asp
            660                 665                 670
Ala Asp Glu Ile Ile Val Leu Asp Gln Gly Lys Val Ala Glu Arg Gly
        675                 680                 685
Thr His His Gly Leu Leu Ala Asn Pro His Ser Ile Tyr Ser Glu Met
    690                 695                 700
Trp His Thr Gln Ser Ser Arg Val Gln Asn His Asp Asn Pro Lys Trp
705                 710                 715                 720
Glu Ala Lys Lys Glu Asn Ile Ser Lys Glu Glu Glu Arg Lys Lys Leu
                725                 730                 735
Gln Glu Glu Ile Val Asn Ser Val Lys Gly Cys Gly Asn Cys Ser Cys
            740                 745                 750
```

INTERNATIONAL SEARCH REPORT

PCT/CN 97/00120

A. CLASSIFICATION OF SUBJECT MATTER

IPC[6] C07K14/47, C12N15/12, C12Q 1/68

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched(classification system followed by classification symbols)

IPC[6] C07K14/47, C12N15/12,C12Q 1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the field searched

Chinese Patnets, Chinese Scientific and Technical Journals

Electronic data base consulted during the international search(name of data base and, where practicable, search terms used)

WPI, MEDLINE, EMBAS, CAplus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant claim No.
A	Hum. Mol. Genet., 5 (10), 1649-1655 (Allikmets,Rando et al) 1996 See Abstract	1-12 16-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason(as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24. March 1998 (24.03.98)	02 APR 1998 (02.04.98)
Name and mailing address of the ISA/ The Chinese Patent Office 6, Xitucheng Road, Haidian District, Beijing, 100088, China Facsimile No. 86-010-62019451	Authorized officer SUN, guangxiu Telephone No. 86-010-62093884

Form PCT/ISA/210(second sheet)(July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN 97/00120

Box I. Observations where certain claims were found unsearchable(Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a)for the following reasons:

1. ☒ Claims Nos.: 13-15
because they relate to subject matter not required to be searched by Authority, namely:
they relate to therapeutic and diagnostic methods.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II. Observations where unity of invention is lacking(Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

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